body (center left), GFP (center right) and Hoescht nuclear stain (right) (scale bar 10 μm). (g) PNA lectin blot analysis (left) and intensity profiles (right) of mucins of varying sizes in extracts of transiently transfected HEK293T cells.

[0014] FIG. **3**: Engineering the Frequency of Glycosylation Sites in the Muc1 Polymer Backbone Tunes O-glycan Maturation. (a) Components and features of secreted Muc1 and engineered variants each with 21 tandem repeats. (b) Tandem repeat sequences of secreted mucin mutants and the molecular weight of the polypeptide backbones. Single, double, and triple glycosylation mutants (sMuc1S, sMuc1D, and sMuc1T) have one, two or three, serine/threonine (S/T) to alanine substitutions per repeat, respectively. The sequences under sMuc1 mutants (21 repeats) are from top down: SEQ ID NO:8, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7 (c) Representative Western blot analysis of affinity-purified recombinant secreted mucins from FreeStyle™ 293-F cell culture media probed with anti-SUMOstar antibody and PNA, s-WGA and VVA lectins (of three independent experiments). The lectin blot was co-stained in multiple colors with PNA-Alexa Fluor 568, s-WGA-FITC, and biotinylated VVA (Secondary: NeutrAvidin-Dylight 650). (d) Representative fluorescence intensity electrophoretograms of the blots in (c). (e) Ratiometric intensity analysis of PNA to VVA signal (upper) and s-WGA to VVA signal (lower) for the indicated mucins and their corresponding frequency of S/T glycosylation sites in the polymer backbone. Ratiometric fluorescence intensity was quantified along each lane and normalized to signal from the secreted mucin with wild-type Muc1 tandem repeats (sMuc1); data presented as the mean and SEM from at least three independent experiments. * $P<0.05$ ** $P<0.01$ *** $P<0.001$ (f) Left: MALDI-TOF mass spectra registered for samples of permethylated glycan alditols from secreted mucins with wild-type Muc1 tandem repeats (sMuc1) and triple mutant (sMuc1T) from HEK293T cell culture media. The ion signals were annotated with respect to the relative masses of molecular ions (m/z) detected as sodium adducts and by assignment of the respective core structure (red for Core 1 and black for Core 2). Right: Schematic presentation of O-linked glycans detected on the secreted mucins.

[0015] FIG. **4**: Designer Mucin Domains Reveal Sequence-Specific Effects on Glycosylation. The sequences shown in FIG. **4** are KEPAPTTP (SEQ ID NO:1) DAATPAP (SEQ ID NO:2) DAATPAPP (SEQ ID NO:3) and PASTSAPG (SEQ ID NO:4). (a) Components and features of designer mucins. (b) Predicted Molecular Weight of the mucin polypeptide backbones. (c) Representatie Western blot analysis (from three independent experiments) of indicated constructs in extracts of transiently transfected HEK293T cells probed with anti-GFP antibody or co-stained with PNA and VVA lectins. (d) Representative Fluorescence intensity electrophoretograms of the western blots in (c) for indicated constructs from three independent experiments. Dashed lines indicate the peak of the glycoform visible in the PNA blot. Shaded boxes indicate the regions between the bands on the anti-GFP blot with the highest and second highest apparent molecular weights. (e) Ratiometric intensity analysis of PNA to VVA staining for the indicated mucins and their corresponding frequency of serine and threonine glycosylation sites in polymer backbone. Fluorescence intensity was quantified along each lane of the dual-probed lectin blot, and the PNA: VVA ratio was normalized to that of the KEPAPTTP (SEQ ID NO:1) x20 mucin; data presented as the mean and SEM from three independent experiments. (f) The fold change in PNA: VVA ratio with doubling the indicated mucin backbone size from 40 to 80 tandem repeats; data presented as the mean and SEM from three independent experiments. * $p<0.05$

[0016] FIG. **5**: Tuning Mucin Glycosylation through Cytoplasmic Tail Engineering. (a) Components and features of cell-surface mucins with synthetic 21-amino-acid transmembrane anchors (TM21) and engineered cytoplasmic motifs; native CT refers to a native cytoplasmic tail adapted from Muc1. (b) Lectin blot analysis of the indicated mucin isoforms from transiently transfected HEK293T cells to detect sialylated O-glycans by periodate oxidation and Core-I structures by PNA; blots are representative of three independent experiments. (c) PNA-lectin blot analysis of the indicated mucin isoforms before and after sialidase treatment; blots are representative of three independent experiments. (d) Top: Representative MAA and PNA lectin blot analysis (from four independent experiments) of the indicated mucin isoforms immunoprecipitated from transiently transfected HEK293T cells. Bottom: Ratiometric intensity of sialic acid to Core 1 glycan signal (MAA: PNA); data presented as the mean and SEM from four independent experiments. * $P<0.05$

[0017] FIG. **6**: Western blot analysis of MCF10A cells edited with lentivirus with native repetitive (Native Muc1) versus codon-scrambled Muc1 cDNAs (Muc1_42).

[0018] FIG. **7**: Mucins with Tunable Sizes. The sequences shown in FIG. **7** are PDTRPAPGSTAPPAHGVTSA (SEQ ID NO:8) (a) Components and features of mucin constructs with GFP reporter, native Muc1 transmembrane anchor, and codon-scrambled Muc1 tandem repeats. (b) Representative immunofluorescence images of transiently transfected HEK293T cells expressing the GFP-tagged Muc1 constructs illustrated in (a) and co-stained with PNA, anti-Muc1 antibody, and Hoechst nuclear stain (scale bar 10 μm) from three independent experiments. (c) Components and features of mucin constructs with synthetic 21-amino-acid transmembrane anchor (TM21) and codon-scrambled Muc1 repeats. (d) Predicted molecular weight for mucin polypeptide backbone illustrated in (c). (e) Representative Western blot analysis (of three independent experiments) of TM21 constructs illustrated in (c) from extracts of transiently transfected HEK293T cells and probed with PNA lectin or anti-Muc1 antibody. (f) Representative phase-contrast images of HEK293 Ts expressed indicated constructs in (c) from three independent experiments (scale bar 100 μm).

[0019] FIG. **8**: Western blot Image of affinity-purified recombinant secreted mucins from FreeStyle™ 293-F cell culture media probed with anti-6× His antibody and VVA lectin

[0020] FIG. **9**: Cell-Surface Mucin Mutants Derived from Muc1 Tandem Repeat Sequences. The sequences shown in FIG. **9** under mMUC1 mutants (21 repeats) from top down are PDTRPAPGSTAPPAHGVTSA (SEQ ID NO:8), PDTRPAPGATAPPAHGVTSA (SEQ ID NO:5) PDTRPAPGATAPPAHGVTAA (SEQ ID NO:6) and PDARPAPGATAPPAHGVTAA (SEQ ID NO:7). (a) Components and features of mucins constructed with 21 native or engineered Muc1 repeats, GFP reporter and native Muc1 transmembrane anchor. (b) Tandem repeats and predicted backbone molecular weight of native Muc1 (mMuc1) or engineered variants with single, double, or triple serine/threonine to alanine substitutions (mMuc1S, mMuc1D, or mMuc1T). (c) Repre-